REMARKS

Claims 1-38, 40, 50, 60, and 80-86 are pending and under consideration in the application. Claims 1, 60, and 83 to 86 have been amended herein. Claim 87 has been added. Claim 10 has been canceled without prejudice or disclaimer. No new matter has been added with the Amendments. The amendment to claims 1 and 60 is supported, for example, by page 34, second full paragraph. The amendment to claims 83 to 86 is a typographical change. Newly added claim 87 is supported, for example, by page 29, line 26 to page 34, line 5. The replacement figures 2A, 2B, 3, 4, 5, 7A, 7B, 9A, and 9B included herein change only formatting of these figures. Upon entry of the Amendment, claims 1-9, 11-38, 40, 50, 60, and 80-87 will be pending.

Specification

The Office Action objects to the specification because it allegedly includes embedded hyperlinks. The specification is amended herein to remove embedded hyperlinks. Accordingly, Applicants respectfully request withdrawal of the objection to the specification.

Claim Rejection under 35 U.S.C. § 112, First Paragraph

Claims 1-38, 40, 50, 60, and 80-86 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly not meeting the written description requirement. Applicants respectfully traverse the rejection. The Office Action asserts that the specification indicates that dissociation of the destabilization domain from the reporter protein is critical for accumulation of the reporter/target protein. The Office Action alleges that the only activity that was demonstrated to effectively dissociate the destabilization domain from the reporter/target was protease activity. Therefore, the Office Action alleges that the specification does not adequately describe the disclosure as filed.

As a preliminary note, Applicants point out that claim 23, and therefore claims dependent therefrom, recite that the linker includes a protease recognition site. Furthermore, claims 38 and 40 do not recite a linker. Accordingly, Applicants respectfully assert that the

rejection of the claims as not adequately described by the specification, is not applicable to claims 23, and claims 24-37, which depend therefrom, as well as claims 38 and 40. Furthermore, independent claims 1 and 60 have been amended herein to recite that the linker includes a recognition motif for a protease. Accordingly, for claims 1 and 60 and claims dependent thereof, Applicants respectfully assert that the rejection has been overcome.

Regarding claim 50 the specification identifies a number of activities in addition to protease activity, that could be used with the present invention (page 11, lines 21-27). Furthermore, the specification discloses that the linker of the invention can include two separate polypeptide chains capable of interacting with each other (page 36, line 3 to page 38, line 4, and page 44, lines 13-31). This approach allows activities, in addition to protease activity, to be assayed that affect the ability of the two polypeptide chains of the linker to interact. Many such polypeptides capable of interacting are known in the art.

The specification provides examples of suitable interaction domains including SH2, SH3, PDZ, 14-3-3, WW, and PTB domains and indicates that additional domains can be identified from the database of interacting proteins available on the worldwide web at doe-mbi.ucla.edu. (Page 36, lines 15-19). A skilled artisan will not only recognize these domains, but also will recognize that it is well established that interaction between these domains can be regulated by various activities. For example, it is well-known in the art that 14-3-3 domains interact with serine and threonine residues on proteins and SH2 and PTB domains interact with tyrosine residues, in a phosphorylation-state dependent manner (See e.g., Couture et al., "Regulation of the Lck SH2 Domain by Tyrosine Phosphorylation," *J. Biol. Chem.*, 271:24880-84 (1996) (Exhibit A). Therefore, it will be understood that a kinase or phosphatase activity, for example, will affect the interaction of these domains with their targets. Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1-38, 40, 50, 60, and 80-86 under 35 U.S.C. § 112, first paragraph as allegedly not meeting the written description requirement.

Claim Rejection under 35 U.S.C. § 112, First Paragraph

Claims 1-38, 40, 50, 60, and 80-86 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly not enabled by the specification. Applicants respectfully traverse the rejection. The Office Action distinguishes embodiments of the present invention performed *in vitro* from those performed *in vivo*. With respect to *in vitro* embodiments, the Office Action alleges that the claims are enabled only to the extent that they read on a chimeric molecule wherein the linker domain includes a protease recognition site. The Office Action bases this allegation on its assertion that the specification only provides examples wherein the recognition motif is a protease recognition motif. The Office Action asserts that since the specification appears to indicate that the dissociation of the destabilization domain from the reporter moiety is critical for accumulation of the reporter, the linker domain must include a recognition motif for an activity that hydrolyzes peptide bonds in order to dissociate the destabilization domain from the reporter.

As a preliminary note, Applicants point out that claim 23, and therefore claims dependent therefrom, recite that the linker includes a protease recognition site. Furthermore, claims 38 and 40 do not recite a linker. Accordingly, Applicants respectfully assert that the rejection of the claims as not enabled by the specification, is not applicable to claims 23, and claims 24-37, which depend therefrom, as well as claims 38 and 40. Furthermore, independent claims 1 and 60 have been amended herein to recite that the linker includes a recognition motif for a protease. Accordingly, for claims 1 and 60 and claims dependent thereof, Applicants respectfully assert that the rejection has been overcome.

Regarding the allegations in the Office Action that the pending claims are only enabled with respect to protease activity, as indicated above, the specification identifies a number of activities, in addition to protease activity, that could be used with the present invention (page 11, lines 21-27). Furthermore, the specification discloses that the linker of the invention can include two separate polypeptide chains capable of interacting with each other (page 36, line 3 to page 38, line 4, and page 44, lines 13-31). This approach allows activities to be assayed that affect the ability of the two polypeptide chains of the linker to interact. As indicated above,

many such activities are listed in the specification and known in the art. Based on these embodiments wherein the linker includes two polypeptide chains that interact, a skilled artisan will recognize that it is not necessary that the activity hydrolyzes peptide bonds. Rather, for these methods any of the many known activities for modulating protein interactions can be used (see e.g., page 11, lines 21-27).

Regarding claims drawn to methods for detecting an activity in cells *in vivo*, the Office Action alleges that the claims are enabled only to the extent that they read on methods wherein the reporter protein is a bioluminescent protein and wherein the linker includes a protease recognition site. As a preliminary note, claims 80-82 recite that the method is performed *in vitro*. Therefore, Applicants respectfully assert that the reasons for rejection related to *in vivo* methods are not applicable to these claims.

The Office Action attempts to support its allegation that the specification only enables *in vivo* methods in which the reporter protein is a bioluminescent protein and wherein the linker includes a protease recognition site, by reiterating its arguments regarding the specification only disclosing a protease activity, included above. Furthermore, the Office Action alleges that in order to detect the activity of the reporter protein in a cell *in vivo*, the reporter protein must be able to be visualized in a living cell. Additionally, the Office Action alleges that the specification does not enable claims drawn to regulating the concentration of a target molecule in a cell *in vivo*, because allegedly the specification does not provide any examples that indicate that the methods of the invention can precisely regulate the concentration of any molecule in a plant or animal.

Regarding the allegations in the Office Action that the pending claims are only enabled with respect to protease activity, as set out above, the specification identifies a number of activities, in addition to protease activity, that could be used with the present invention (page 11, lines 21-27). Furthermore, the specification discloses that the linker of the invention can include two separate polypeptide chains capable of interacting with each other (page 36,

line 3 to page 38, line 4, and page 44, lines 13-31). This approach allows activities to be assayed that affect the ability of the two polypeptide chains of the linker to interact. As indicated above, many such activities are listed in the specification and known in the art. Based on these embodiments wherein the linker includes two polypeptide chains that interact, a skilled artisan will recognize that it is not necessary that the activity hydrolyzes peptide bonds. Rather, for these methods any of the many known activities for modulating protein interactions can be used (see e.g., page 11, lines 21-27).

Regarding the allegation in the Office Action that in order to detect the activity of the reporter protein in a cell *in vivo*, the reporter protein must be visualized in a living cell, the Applicants respectfully assert that a reporter moiety expressed *in vivo* can be detected using a number of techniques, even if the reporter moiety is not a bioluminescent protein. First, the reporter moiety can be detected in cells that express the reporter *in vivo*, by isolating the cells from an organism and then detecting the reporter. Many different types of assays can be performed on isolated cells to detect the reporter, even if the reporter is not a bioluminescent protein. For example a fluorescent substrate capable of passing across the cell membrane, such as CCF2/AM, can be used and detected using flow cytometry. (See e.g., Example 8). Alternatively, as another example, the isolated cells can be lysed and enzymatic activity can be analyzed (See e.g., page 76, lines 20-29). Furthermore, the reporter moiety can be detected by analyzing lysates of tissues that express the reporter moiety (See e.g., Tsirigotis et al., *BioTechniques*, 31:120-130, 127, left column (2001); and Hondred et al., *Plant Physiology*, 119, 713 (1999), both of record in the present case). For example, Hondred et al. teach that chimeric ubiquitin protein fusions can be detected in tobacco when coupled to a number of reporter moieties and target proteins including glucuronidase, luciferase, amylase, and acyl carrier protein (See Figs 3-6). Furthermore, methods are available and known in the art that provide for detection of a reporter moiety expressed *in vivo*, for a bioluminescent ant non-bioluminescent reporter moieties without cell isolation. For example, reporters that provide for *in vivo* selection can be utilized, such as a DHFR protein that can be used to render a transgenic plant resistant to MTX (See e.g., Bachmair et al., *Proc. Natl. Acad. Sci.*, 90:418-21 (1999)).

Accordingly, Applicants respectfully assert that numerous methods for detecting a reporter moiety are available that can be used even when a method of the invention is performed such that a cell is provided *in vivo*, that do not require a bioluminescent or fluorescent reporter moiety.

Regarding the allegation that claims drawn to regulating the concentration of a target molecule in a cell *in vivo*, are not supported because the specification allegedly does not provide any examples that indicate that the methods of the invention can precisely regulate the concentration of any molecule in a plant or animal, Applicants respectfully assert that one of the advantages of the present invention is that it allows for the careful regulation of protein concentration by controlling the number of ubiquitin moieties included (Page 5, lines 5-15). As indicated in the specification, by varying the number of destabilization domains present in the multimerized destabilization domain, it is possible to titrate the degree of destabilization, and therefore the steady state concentration of the target protein within a cell or transgenic organism (See e.g., Page 45, lines 21-30). Therefore, depending on whether the desired protein concentration in an organism is relatively high or relatively low, the number of copies of the destabilization domain can be determined (Page 60, lines 3-7). Furthermore, the specification provides experimental evidence of the ability to control protein concentration based on the number of destabilization domains included (Example 9, which starts on page 78). Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1-38, 40, 50, 60, and 80-86 under 35 U.S.C. § 112, first paragraph as allegedly not enabled by the specification.

It is also noteworthy that newly added claim 87 recites that the method is performed *in vivo* and the reporter is a bioluminescent protein or a fluorescent protein. Therefore, Applicants respectfully assert that claim 87 even further overcomes the rejection.

In view of the amendments and the above remarks, it is submitted that the claims are in condition for allowance and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

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EXHIBIT A

Copy of Couture et al., "Regulation of the Lck SH2 Domain by Tyrosine Phosphorylation," *J. Biol. Chem.*, 271:24880-84 (1996).